DISTRIBUTION AND BINDING OF DANTROLENE SODIUM IN WHOLE BODY SECTION AND FIXED ISOLATED ORGAN—FLUORESCENT OBSERVATION

PARVINE NOUHNEJADE AND SHOHREH MALEKI

*Department of Biology, Pharmaceutical Research Center, Darou-Pakhsh
Pharmaceutical Co., Darou-Pakhsh Ave., Karaje Road,
P.O. Box 13/185-877, Tehran, Iran*

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Distribution and localization of dantrolene-Na in the organs of mice has been studied, using whole body frozen sections by the aid of fluorescent macro- and microscopic techniques. The results obtained in these experiments were compared to the tissues prepared by wax-embedding techniques. Uptake of the drug by isolated rat organs and dissected fresh locust thoracic muscle has also been examined.

Based on present studies, dantrolene-Na was not distributed uniformly throughout the body. It was mainly accumulated in intestine and liver and partly in kidneys. Dantrolene accumulation was not dose-dependent. However, length of administration time was quite effective on the pattern of distribution. At shorter intervals it was mainly accumulated in intestine, liver and slightly in kidney. Longer exposure to the drug resulted in more intense accumulation in intestine and liver.

Comparison of frozen and fresh material to fixed sections further supports the previous investigations that fixation procedures grossly alter the normal state of tissues and inevitably give rise to artifactual observations.

Dantrolene sodium (Dantrium) is a hydantoin derivative which is an effective muscular relaxant, (3) and acts by inhibition of Ca^{2+} release from sarcoplasmic reticulum (SR) (7).

Several lines of evidence indicated the histopathological alterations related to the long period administration of the drug (10, 11, 12, 14, 6, 1).

Dantrolane exhibits fluorescent properties with an excitation of 395 nm and fluorescence of 530 nm (9, 5).

Spectrophotofluorometric studies have revealed the binding of dantrolene to SR of rabbit skeletal muscle (5). However, microscopic data on the binding of the drug to SR and its distribution in internal organs of live animals is not available.

The aim of the present study was to clarify the distribution of dantrolene-Na in the body of live animals as a function of dosage and application time. As a control we have used wax-embedded tissues to evaluate the validity of fluorescent binding patterns. Drug uptake by isolated rat organs and its binding to SR denuded locust fresh myofibrils have been microscopically investigated.

MATERIALS AND METHODS

Dantrolene-Na dissolved in PEG-400 to the final concentration of 6.25, 12.5 and 25 mg/kg of body weight was injected intraperitoneally to either sex of mouse of albino strain with an average of 19 g body weight. Controls were taken using uninjected animals. After post injection intervals of 1/2, 1, 3, 4 and 5 hr animals were deeply anaesthetized by putting them into a closed container containing diethylether vapour and then taken into the procedure as follows:

(a) *Whole Body Sections*

The animals were embedded in carboxymethylcellulose (LKB, Sweden), and were frozen by immersion into the liquid nitrogen. Whole body tape sections of 60 μm were taken on a LKB, PMV cryostat at -20°C . Sections were kept at -10°C inside the instrument, allowing them to dry and then were transferred to room temperature. Fluorescent substance was traced by exciting the sections with a HBO 50 lamp (Zeiss Co.). Examination of the tapes was carried out by the aid of setting appropriate barrier and exciting filters (described in b) to the camera. Agfapan Vario-XL was used for photographic registration. To identify the location of organs in whole body sections tapes were stained by hematoxyline-eosine techniques.

(b) *Fixed Materials*

After sacrificing the animals, different internal organs including liver, intestine, kidneys, brain and skeletal muscle were fixed in 10% neutral formalin and then processed through paraffin wax embedding procedure and sectioned at 6 μm on a Reichert-Jung rotary microtome.

In a separate experiment, isolated organs of rat were incubated in 0.9% NaCl solution containing dantrolene-Na to the final concentration of 50 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$ for 1/2 hr. Tissues were then fixed in formalin and sectioned at 6 μm . Slides were mounted in glycerol and examined under a Zeiss Photomicroscope III equipped with HBO 50 lamp, excitation filters of BP 400–440 and barrier filters of LP 470.

(c) *Fresh Locust Muscle*

Locust thoracic muscles were dissected and incubated in 0.9% NaCl solution containing dantrolene-Na to the final concentration of 25 $\mu\text{g}/\text{ml}$ for 1/2 hr and then smeared on a microscopic slide. Myofibrils denuded of SR were prepared by washing the SR off according to the method of Bullard *et al.* (1973). Slides were observed with Photomicroscope III.

RESULTS

It has previously been reported that only 20–25% of dantrolene oral administration is absorbed and the intestine is the main site of drug absorption (9). We therefore decided to administer the drug by intraperitoneal injection to have the maximum rate of absorption. We started by injecting 25 mg/kg of animal body weight as had been cited as a minimal dose for skeletal muscle relaxation in rats (8). But using this concentration on mouse manifested the failure of respiratory system which eventually gave rise to some degree of paralysis and general acute toxicity resulting in the production of ascitic fluid in the peritoneal cavity. We therefore

FIG. 1. The view of whole body section (WBS) of mice treated with 12.5 mg/kg of dantrolene-Na for 1 hr.

used a single dose of 12.5 mg/kg of body weight which had no toxicity and gave a good fluorescent pattern.

Figure 1 represents the pattern of dantrolene distribution in mice after 1 hr of drug administration. Dantrolene is mainly concentrated in the intestine and gradually penetrates into the liver. A slight diffusion of the drug into the kidneys could also be traced. Figure 2, which is the view of drug accumulation after 5 hr, shows a higher concentration of the drug in intestine and liver. A high degree of fluorescent staining could be observed in the bladder. Muscles also show a slight fluorescence at this time. Control sections show only an insignificant staining of stomach and intestine. There is a red fluorescence (in contrast to green fluorescence of dantrolene) in the arderian gland (Fig. 3).

The results obtained with fixed materials, either the animals receiving drug injection, or separated organs incubated in dantrolene is somewhat confusing. There is a very high background fluorescence in both treated and control sections. Figure 4, which is a control section of gall bladder surrounded by liver tissues, strongly fluoresces in green. Figure 5 is a liver section taken from untreated animal, showing a moderate fluorescence in hepatocytes of centrilobular area; whereas Figure 6 which is a section from liver incubated in 10 $\mu\text{m}/\text{ml}$ of dantrolene, shows fluorescence staining of liver cells to the same degree. Figures 7 and 8 represent control sections of renal cortex and kidney incubated in 50 $\mu\text{g}/\text{ml}$ of drug respectively, which both show the same degree of green non-specific staining.

Figures 9 and 10 show the fluorescence binding of dantrolene to locust thoracic muscle. Isolated myofibrils with distinctive Z-lines show far less degree of fluo-

FIG. 2. The view of WBS of mice treated with 12.5 mg/kg of dantrolene-Na for 5 hr.

FIG. 3. Control WBS.

FIG. 4. Control section taken on cryostat viewing the gall bladder.

rescent than the bulk of muscle which is not denuded of SR. Smeared muscle containing SR shows a very uniform and high fluorescence binding.

DISCUSSION

The data presented in this article supports the previous findings by Dykes (7), that the intestine is the major site of dantrolene action. At a 1 hr exposure interval, dantrolene appears in the intestine and at the same time penetrates into the liver and kidneys. After 5 hr it completely fills the liver, being found in bladder and lightly in muscles.

The existing data on dantrolene metabolism indicates that the half life of this drug is about 8.7 hr, and the prime site of drug absorption is the small intestine (7). In agreement with this view, dantrolene first appears in the small intestine at 1 hr and gradually accumulates in this organ after 5 hr of exposure to the drug. However, the existence of moderate staining at this site indicates that some of this fluorescence is due to tissue autofluorescence.

The liver is known to be the major site of drug metabolism (13) and this is probably true for dantrolene. The increase of drug accumulation in the liver from 1 to 5 hr after drug administration may imply that the liver plays some important role in the drug metabolism. Although the possibility of some degree of drug metabolism in the intestine cannot be ruled out, at this stage of experiments the exact metabolic pathway of drug may not be clearly identified.

The other point of interest is that increment of drug concentration does not change the pattern of distribution and the only important factor in drug metabolism is the length of administration time.

The results obtained with fresh locust muscle confirms the biochemical data given by Dehpour and Mahmoudian (4), that the binding site of dantrolene in the muscle is in fact the SR. Muscle fibers denuded of SR show a significant reduction of fluorescent binding (arrow heads).

As other authors, we also came to the conclusion that fixation-embedding procedure grossly alters the structure of tissues and makes the results unreliable. The point that the control sections fluoresce as well as treated tissues strongly favors the use of frozen sectioning technique in histological experiments.

FIG. 5. The view of control section taken from liver preserved by wax-embedding technique. $\times 25$

FIG. 6. Liver tissue incubated in $10\ \mu\text{m}$ for $1/2$ hr, paraffin embedded. $\times 25$

FIG. 7. Control section of renal cortex, paraffin embedded. $\times 25$

FIG. 8. Renal tissue incubated in drug solution ($50\ \mu\text{m}$ for $1/2$ hr), paraffin embedded. $\times 25$

FIG. 9. The view of unfixed locust thoracic myofibrils incubated in $25\ \mu\text{m}$ dantrolene-Na. Note the intensity of fluorescent staining in myofibrils with SR and the decrease of fluorescent in the areas of single myofibres.

FIG. 10. Phase contrast view of Fig. 9. $\times 900$

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